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Rapid Determination of Silver in Nanobased Liquid Dietary Supplements Using a Portable X-ray Fluorescence Analyzer

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ABSTRACT: This paper reports a rapid and straightforward method for the quantitation of total Ag content in nanobased commercially available liquid dietary supplements using a portable X-ray fluorescence (pXRF) analyzer. Figures of merits were evaluated by analyzing a series of $AgNO_3$ standards. This method was shown to have a detection limit of 3 ppm, a quantitation limit of 10 ppm, and a broad linear range from the detection limit to 10000 ppm (1%). Accurate detection and quantitation of Ag content in well-characterized Ag nanoparticle samples and in nanobased liquid dietary supplements were achieved with good correlation (i.e., percentage difference average values under 15%) between the total Ag concentration obtained by the pXRF analyzer and by inductively coupled plasma mass spectrometry (ICP-MS). Furthermore, accurate quantitation of Ag in the presence of high concentrations of potential spectral interferences was also demonstrated.

KEYWORDS: *nanoscale silver, dietary supplements, X-ray fluorescence analyzer, nanomaterials, inductively coupled plasma mass spectrometry, quantitation*

INTRODUCTION

The number of commercially available dietary supplements that claim to contain nanoscale ingredients has grown at a rapid pace over the past few years. These nanobased supplements range from vitamins to weight-loss pills and sports performance enhancers.[1] As defined in the Dietary Supplement Health and Education Act of 1994 (DSHEA, an amendment to the Federal Food, Drug and Cosmetic Act), the term "dietary supplement" refers to a product (other than tobacco) that, among other things, (1) is intended for ingestion, (2) is labeled as a "dietary supplement", and (3) is intended to supplement the diet and contains one or more of the following ingredients: vitamins, minerals, herbs or other botanicals, amino acids, dietary substances for use by man to supplement the diet by increasing the total dietary intake, or concentrates, metabolites, constituents, extracts, or combinations of these ingredients.[2] Under the DSHEA, the dietary supplement manufacturer is responsible for ensuring that the product is safe before it is marketed and that any claims made are substantiated by evidence to show that they are not misleading or false. The U.S. Food and Drug Administration (FDA) is responsible for taking action against any unsafe dietary supplement after it reaches the market.[3]

Nanoscale Ag, for example, is an ingredient often found in nanobased dietary supplements due to its antimicrobial properties.[4] Nevertheless, the use of nanoscale materials such as Ag in FDA-regulated products, especially products intended for human consumption, is of particular concern because of the unknown impact on consumer health and safety. It has been well documented that ingestion or inhalation of large doses of bulk Ag has detrimental effects on human health, such as damage to the gastrointestinal tract, upper and lower respiratory irritation, and discoloration of the skin (i.e., argyria), among others.[5−7] It is important, for the protection of consumers, that the FDA develops screening protocols which could be rapidly transferred and utilized for monitoring nanoscale Ag in dietary supplements in the event that toxicological studies indicate an increased risk associated with the intake of this nanomaterial. Such screening methods would eventually be followed by studies on methodologies to confirm the size and chemical composition of the nanoscale Ag.

Screening methodologies based on X-ray fluorescence (XRF) spectrometry have shown great promise, due to the technique's simplicity, high sample thoughput, and minimal sample preparation.[8] The potential of using a portable X-ray fluorescence (pXRF) analyzer to screen for elements of interest in food, artwork, soils, archeological samples, and environmental materials, among others, has been reported.[9,10] pXRF analyzers have been successfully used to screen for common toxic elements, such as Pb, As, and Cd, at parts per million (ppm) levels within FDA-regulated products;[10−14] however, the ability of a pXRF analyzer to identify the chemical composition and quantitate engineered nanomaterials, such as nanoscale Ag, has not been fully explored.

We report the use of a pXRF analyzer as a rapid, nondestructive, and accurate screening technique for the detection of Ag in well-characterized Ag nanoparticle samples of different sizes and within commercially available nanoscale Ag-based dietary supplements. The results obtained using a pXRF analyzer were confirmed and validated using inductively coupled plasma mass spectrometry (ICP-MS). The presence of nanoscale Ag within the supplements was demonstrated by transmission electron microscopy (TEM). One of the most attractive features of the reported method is its high sample throughput due to the very fast analysis times achievable with a pXRF analyzer. With the time required for sample preparation as well as the time

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for the analysis of standards and samples (i.e., 120 s) taken into consideration, throughputs of at least 20 samples per hour can be easily achieved. Although pXRF spectrometry is not a technique capable of distinguishing between ionic, bulk metallic, and nanoscale Ag, it is a valuable technique for the rapid screening and quantification of elemental content within consumer products by field investigators and laboratory analysts. The utilization of screening methodologies allows for greater efficiency and sample throughput prior to performing more specialized and time-consuming confirmatory methodologies, such as electron microscopy, for nanomaterials within consumer products.[15−17] Analysis of liquid dietary supplements using a pXRF analyzer is faster and simpler than with traditional analytical methods such as ICP-MS, which makes the pXRF analyzer a powerful tool in the screening of larger numbers of supplements for analytes of interest.

MATERIALS AND METHODS

Reagents and Materials. Nitric and hydrochloric acid (Optima grade) were purchased from Fisher Scientific (Houston, TX, USA). Ag (10000 and 1000 ppm), Cd (1000 ppm), Pd (1000 ppm), Se (1000 ppm), and In (10 ppm) single-element ICP-MS standards were acquired from Ricca Chemical Co. (Arlington, TX, USA), Spex CertiPrep Group (Metuchen, NJ, USA), and Inorganic Ventures (Christiansburg, VA, USA). Multielement ICP-MS standard solution 2 (10 ppm Ag, Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Tl, U, V, Zn) was purchased from Spex CertiPrep Group. Type I ultrapure water (18.2 MΩ·cm) was used for all solution preparations.

Polypropylene X-ray film and 32 mm plastic XRF sample cups were acquired from Premier Lab Supply (Port St. Lucie, FL, USA). Isopropyl alcohol (70%), from Cumberland Swan (Smyrna, TN, USA), and 300-mesh carbon-coated copper grids, from Electron Microscopy Sciences (Hatfield, PA, USA), were used to prepare samples for analysis by TEM.

Samples. Well-characterized Ag nanoparticles (10, 75, and 110 nm in diameter), stabilized by sodium citrate, were acquired from nanoComposix, Inc. (San Diego, CA, USA). Twelve single- and multiple-element dietary supplements that claim to contain nanoscale Ag were purchased from various Internet vendors.

Inductively Coupled Plasma Mass Spectrometry. Analysis of standards, matrix spikes, and samples by ICP-MS was performed according to the method reported by Mudalige and Linder.[18] Briefly, samples were first digested using a CEM (Matthews, NC, USA) MARS 5 microwave-accelerated reaction system (CEM MARSXpress Teflon vessels; CEM MARSXpress vessel capping station) and then analyzed by an Agilent Technologies (Santa Clara, CA, USA) 7700x ICP-MS (Micro mist nebulizer; autosampler ASX-500; MassHunter workstation software version A.01.02) under no-gas mode. The results obtained by ICP-MS were compared to the data acquired with the pXRF analyzer, and percentage difference values were calculated and used to evaluate the accuracy of the pXRF analyzer data.

Transmission Electron Microscopy. Samples for TEM analysis were prepared by adding 2 μL of the test article (i.e., well-characterized nanoparticle samples and dietary supplement products) and 2 μL of isopropyl alcohol on 300-mesh carbon-coated copper grids and allowing the samples to dry. All micrographs were collected with a JEOL (Peabody, MA, USA) GEM 2100 transmission electron microscope equipped with a LaB_6 electron source and operated at an acceleration voltage of 200 kV.

X-ray Fluorescence Spectrometry. *Working Standards.* Ionic Ag working standards were prepared by serial dilution using a 10000 ppm $AgNO_3$ ICP-MS standard solution as the stock. Each working standard was diluted to the appropriate Ag concentration (1.0, 2.5, 5.0, 10, 25, 50, 100, 250, 500, 1000, 2500, and 5000 ppm) with type 1 ultrapure water and nitric acid to give a final acid concentration of 3% (w/w). Working standards were prepared in class A 10 mL volumetric flasks and then transferred to 32 mm XRF sample cups for analysis.

Sample Preparation and Analysis. Samples for XRF analysis were freshly prepared immediately prior to analysis by transferring 9.0 mL of the standard/sample solution (i.e., enough volume to fill the XRF sample cup) to a plastic XRF sample cup and by sealing the cup with a thin polypropylene film and securing it with a snap-on ring. Each solution was mixed well prior to being transferred to a sample cup. All ionic Ag standards, well-characterized Ag nanoparticle samples, and nanoscale Ag dietary supplements were analyzed using an Olympus (Waltham, MA, USA) Innov-X X-5000 pXRF analyzer (50 kV, 200 μA X-ray tube with a Ta anode configuration). A Ta anode configuration provides excellent sensitivity for the analysis of Ag and other transition metals, whereas the use of an Ag tube target could potentially lead to poor results because of higher background counts from elastic scattering. For analysis, the plastic cup containing the sample of interest was placed on top of the probe window, with the side containing the polypropylene film in contact with the window. Similar types of films, such as Mylar, could also be used for analysis. Samples were analyzed using "soil mode" with a beam energy of 50 kV (beam 1), number of replicate measurements = 7, and measurement time = 120 s. Correction values were obtained from an initial empirical calibration (using ionic Ag standards with concentration varying from 0 to 1000 ppm), assigned in the software and automatically applied to all subsequent measurements. With the specific XRF analyzer used in this study, a value of −17 was used as the offset and 0.726 was used for the response factor (i.e., slope correction). All reported concentration values refer to mass fraction, that is, mg/kg (ppm).

Quality Control Parameters for XRF Analysis. A 316 stainless steel standardization coupon, provided by Innov-X, was used to perform energy calibration verification (ECV) of the pXRF analyzer. ECV was performed at least twice per analytical batch: at instrument start-up, at the end of each batch, and at any other time when the instrument detected significant drift.[19] A plastic XRF sample cup packed with SiO_2 was used as the instrument blank (IB). The IB was tested at the beginning and end of each analytical batch and once every 20 samples. The method blank (MB) was prepared by adding 9.0 mL of a 3% nitric acid solution to a plastic XRF sample cup. The MB was analyzed once per analytical batch. An initial calibration verification (ICV) sample, consisting of 100 ppm Ag in 3% nitric acid, was prepared from a second source of $AgNO_3$ and analyzed at least once per analytical batch. Continuous calibration verification (CCV) and precision verification (PV) were performed by analyzing a 100 ppm Ag working standard. The CCV sample was tested once every 20 samples, and the PV sample was analyzed at least once per batch by conducting seven replicate measurements of the sample. ICP-MS multielement standard solution 2, which contains 10 ppm Ag, was used as a certified reference material (CRM). The reported Ag concentration for this CRM is traceable to NIST standard reference material 3151. The CRM was used as a confirmatory sample and was tested at least once per batch. A typical analytical batch consisted of the following samples: ECV, IB, MB, ICV, CCV/PV, CRM (sample 1), samples 2−20 (e.g., matrix spikes, dietary supplements, etc.), IB, CCV, samples 21−40, IB, CCV, and ECV. ICV and CCV values determined by the pXRF analyzer should be within ±20% of their nominal values. Experimental values for the CRM should be within 2 standard deviations of the value published in the Certificate of Reference Material datasheet.

Statistical Analysis. An empirical calibration was performed in addition to the instrument's factory calibration (which consists of Compton normalization for "soil mode") and was used to calculate the pXRF analyzer's correction values (i.e., response factor and offset). The $AgNO_3$ working standards were analyzed with the pXRF analyzer and with ICP-MS, and the calibration curve was generated using linear regression

$$y = mx + b \quad (1)$$

where x is the nominal Ag concentration (in ppm) of the $AgNO_3$ standards and y is the Ag concentration (in ppm) reported by the pXRF analyzer. The values for the slope of the curve, m, and the y intercept, b, were used to calculate the "response factor" and the "offset" correction values, respectively. The coefficient of determination

(r^2) for the calibration curve was >0.999 for Ag concentrations up to 10000 ppm.

Percentage difference values (%DV) were defined as

$$\%DV = \left(\frac{[Ag]_{pXRF} - [Ag]_{ICP\text{-}MS}}{\frac{[Ag]_{pXRF} + [Ag]_{ICP\text{-}MS}}{2}} \right) \times 100 \quad (2)$$

where $[Ag]_{pXRF}$ is the corrected Ag concentration reported by the pXRF analyzer and $[Ag]_{ICP\text{-}MS}$ is the Ag concentration determined by ICP-MS.

The instrument's accuracy was evaluated by analyzing aqueous samples spiked with different concentrations of $AgNO_3$ and by calculating the percentage recovery value (%RV), which was defined as

$$\%RV = \left(\frac{[Ag]_{pXRF}}{[Ag]_{nominal}} \right) \times 100 \quad (3)$$

where $[Ag]_{pXRF}$ is the corrected Ag concentration reported by the pXRF analyzer and $[Ag]_{nominal}$ is the manufacturer's certified value for the Ag concentration of the standard.

Relative standard deviation, expressed as a percentage (%RSD), was calculated to evaluate the instrument's precision:

$$\%RSD = \left(\frac{SD}{\overline{[Ag]}_{pXRF}} \right) \times 100 \quad (4)$$

SD and $\overline{[Ag]}_{pXRF}$ are the standard deviation and the mean of the Ag concentration reported by the pXRF analyzer, respectively.

The method detection limit (MDL) was defined as 3 times the standard deviation of the calibration standard with the lowest analyte concentration. We defined the method quantitation limit (MQL) as 10 times the standard deviation of the calibration standard with the lowest analyte concentration.[20]

RESULTS AND DISCUSSION

A representative XRF spectrum of a 50 ppm $AgNO_3$ standard solution (in 3% nitric acid) is shown in Figure 1. The peak

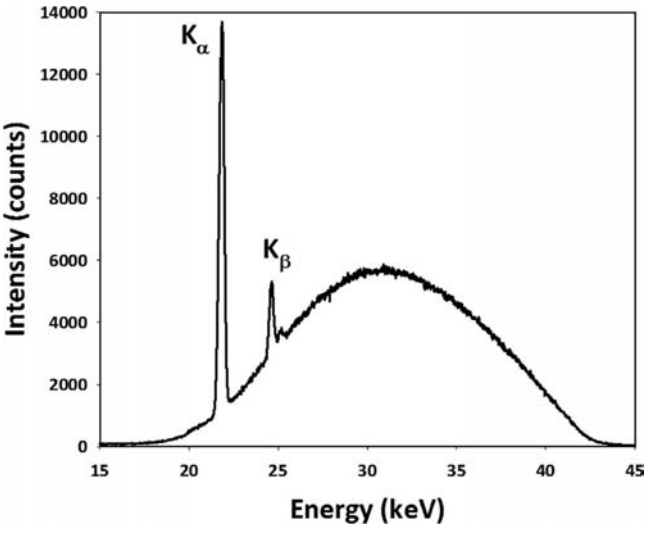

Figure 1. Representative XRF spectrum of a 50 ppm $AgNO_3$ standard solution (in 3% nitric acid) showing the Ag Kα (Kα_1, 22.16 keV; Kα_2, 21.99 keV) and Kβ (Kβ_1, 24.94 keV; Kβ_2, 25.46 keV) X-ray lines.

observed around 22 keV corresponds to the Ag Kα XRF lines (Kα_1, 22.16 keV; Kα_2, 21.99 keV), whereas those observed around 25 keV are due to the Ag Kβ XRF lines (Kβ_1, 24.94 keV; Kβ_2, 25.46 keV).[21] Ideally, the presence of Ag in an unknown sample should be confirmed by the direct concentration readout from the pXRF analyzer as well as by the presence of at least two XRF emission lines for the analyte. Due to a small spectral contribution from Ag impurities in the pXRF analyzer used for these studies, false-positive readings for Ag occurred during the analysis of reagent blanks and samples that did not contain Ag. To minimize the number of false positives obtained with the analyzer and to get more accurate concentration readings for samples containing Ag, we performed an empirical calibration by testing $AgNO_3$ standards of concentrations varying from 1 to 1000 ppm. The Ag concentration calculated by the pXRF analyzer was compared to the nominal concentration for each standard by plotting the Ag concentration obtained by the pXRF analyzer on the y-axis and the nominal concentration of the prepared standards on the x-axis. Correction values were calculated from the linear best fit for the data. The "response factor" correction value was calculated as the inverse of the plot's slope (m), whereas the "offset" correction value was defined as minus the ratio of the y-intercept (b) to the slope (i.e., $-b/m$). These correction values were entered directly into the analyzer, and the software automatically used these values to correct all subsequent determinations of Ag concentration. With the portable XRF analyzer that we used for our studies, values of 0.726 and −17 were used as the "response factor" and "offset" correction values, respectively. All reported Ag concentration values represent the data calculated directly by the instrument's software using the correction values listed above.

Determination of the Analytical Figures of Merit Using Ionic Ag Standards. *Linear Range.* The linear range of the method was evaluated by measuring ionic Ag standards over a wide range of concentrations. Figure 2 shows a

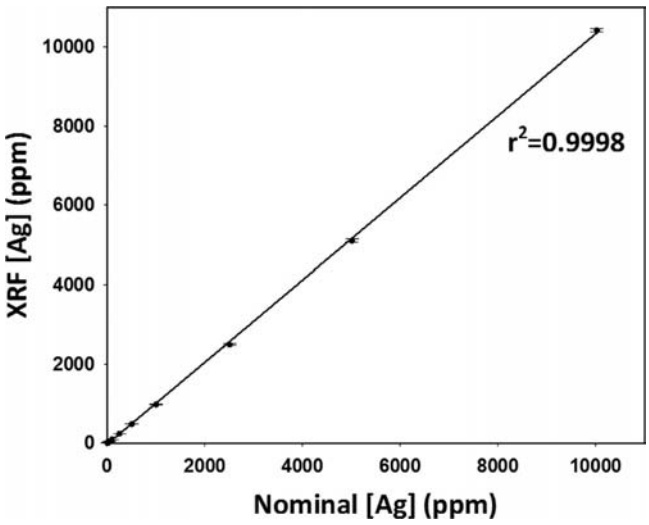

Figure 2. Analysis of $AgNO_3$ standards for the determination of the linear range of the pXRF analyzer.

representative calibration curve for Ag. Values reported on the y-axis, which are determined automatically by the analyzer's software, were calculated as the mean of the corrected Ag concentrations of seven replicate measurements for each ionic Ag standard. The curve shows excellent linearity, as evidenced by an r^2 value of 0.9998. The analyzer exhibits a wide linear range, which spans nearly 4 orders of magnitude, from the MDL up to 10000 ppm (1%).

Precision, Accuracy, Detection Limit, and Quantitation Limit. Evaluation of the method's precision and accuracy is

crucial for the quantitative analysis of FDA-regulated products. The precision of the method was evaluated by testing ionic Ag standards with low (10 ppm), intermediate (100 and 1000 ppm), and high concentrations (10000 ppm) of analyte. The relative standard deviation (RSD) of the mean Ag concentration for each standard was used as the basis for precision assessment. Seven replicate measurements of each standard were performed to calculate the mean RSD, expressed as a percentage, and the values are reported in Table 1. EPA Method 6200 establishes

Table 1. Evaluation of the Method's Precision (n = 7)

sample description	nominal [Ag] (ppm)	%RSD
10 ppm Ag working standard	10	7
100 ppm Ag working standard	100	2
1000 ppm Ag working standard	1000	0.4
10000 ppm Ag stock solution	10000	0.4

that the %RSD should be ≤20% for data to be considered as adequately precise.[19] All of the reported %RSD values for our method were equal to or better than 2% for concentration values above the MQL and well below 10% for all studied concentration values and, therefore, easily met the EPA Method 6200 criteria. For applications where lower %RSD values are required, the precision of the method could be improved by increasing the measurement time.

The method's accuracy was assessed by analyzing Ag matrix spikes of various concentrations and by calculating the mean percentage recovery values, which are reported in Table 2.

Table 2. Analysis of the Ionic Ag Matrix Spikes (n = 7)

nominal [Ag] (ppm)	mean %RV
10	102 ± 9
25	95 ± 5
50	97 ± 2
100	96 ± 1
250	97.7 ± 0.8
500	97.0 ± 0.7
1000	98.4 ± 0.4
2500	100.2 ± 0.3
5000	101.3 ± 0.3

The mean %RV for Ag concentration level ranged from 95 to 102%, which demonstrates that the analyzer is able to accurately measure Ag content in aqueous solutions.

Additional validation of our method was performed by testing a commercially available multielement CRM that contains 10 ppm ionic Ag. The mean Ag concentration reported by the pXRF analyzer (10 ± 1 ppm) fell within the established acceptance criteria of 9.87−10.07 ppm, which represents 2 times the standard deviation of the certified Ag concentration of the CRM (9.97 ± 0.05 ppm). The accurate determination of Ag in this CRM, which contains additional elements that could potentially interfere with the analysis, demonstrates that our method is valid for the detection and quantitation of total Ag content in aqueous matrices. The MDL and MQL for Ag were also determined to be 3 and 10 ppm, respectively.

Selectivity: Impact of Potential Spectral Interferences. Because XRF is a multielement detection technique, spectral overlaps of emission lines of adjacent elements might affect the technique's selectivity. The most common spectral interference in XRF, called Kα/Kβ, involves the overlap of the Kβ X-ray line of element $Z-1$ with the Kα line of element Z (where Z is the atomic number).[19] The Kα/Kβ intensity ratio for a given element is typically greater than 5:1; therefore, the presence of the interfering element $Z-1$ at large concentrations might cause problems.[19]

For the determination of Ag, key potential spectral interferences would be the emission lines from Pd and Cd, as shown in Figure 3. To determine the effect of potential spectral

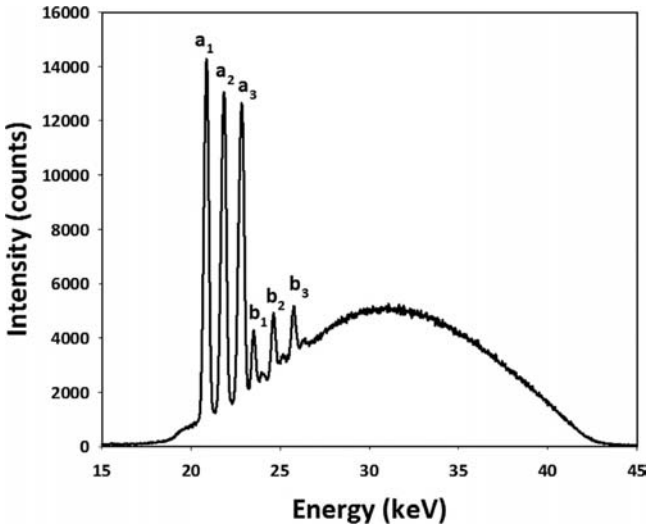

Figure 3. Representative XRF spectrum of a 50 ppm $AgNO_3$ standard solution that contains 500 ppm Cd and 500 ppm Pd, showing the (a) Kα and (b) Kβ X-ray lines for (1) Pd, (2) Ag, and (3) Cd. The Ag peaks showed intensity values higher than those expected for a sample containing 50 ppm Ag due to the spectral contribution from Ag impurities present in the analyzer.

interferences from Pd and Cd on the accuracy of Ag quantitation, we analyzed sample spikes of 50 ppm Ag that also contained Pd, Cd, or both, at a concentration 10 times higher than that of Ag. The results for these measurements are summarized in Table 3. All mean percentage recovery values

Table 3. Evaluation of Potential Spectral Interferences for Ag (n = 7)

sample description	mean %RV
50 ppm Ag	97 ± 2
50 ppm Ag; 500 ppm Cd	82 ± 4
50 ppm Ag; 500 ppm Pd	90 ± 10
50 ppm Ag; 500 ppm Se	97 ± 3
50 ppm Ag; 500 ppm Cd; 500 ppm Pd	100 ± 20

stayed within ±20% of their nominal values, demonstrating that our method is able to accurately determine Ag concentration, even in the presence of an excess of potential interfering elements that are not commonly found in dietary supplements at such high concentrations.

The potential effects of sum peaks in Ag quantitation were also studied. Sum peaks occur when two or more photons arrive at the detector simultaneously and are thus read and converted into one pulse with energy equal to the sum of the photons. Sum peaks due to Se Kα emission lines (Kα_1, 11.18 keV; Kα_1 + Kα_1 sum peak, 22.36 keV)[21] could potentially interfere with the Ag Kα_1 peak. To assess the potential effect of high concentrations of Se in the quantitation of Ag, sample spikes of 50 ppm

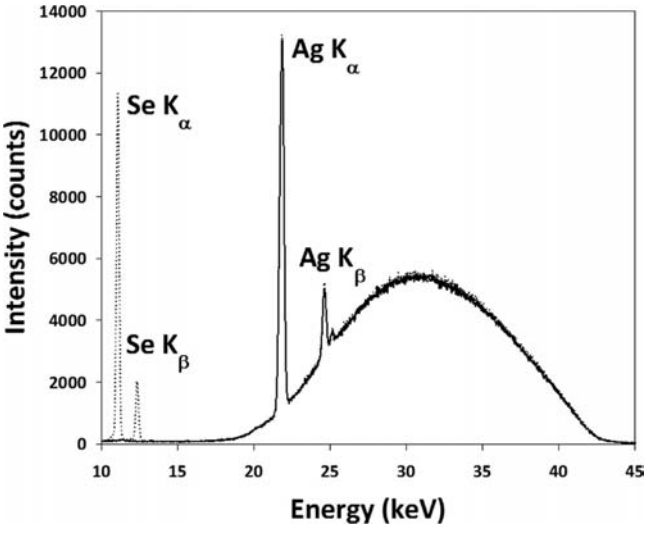

Figure 4. Representative XRF spectrum of a 50 ppm $AgNO_3$ standard solution that contains 500 ppm Se (dotted line), showing the Kα and Kβ X-ray lines for Ag and Se. The XRF spectrum for the 50 ppm $AgNO_3$ standard solution without Se is also shown (solid line) for comparison purposes.

Ag that contained 500 ppm of Se were analyzed. The results, shown in Figure 4 and Table 3, suggest that the presence of high levels of Se in the sample does not interfere with the accurate quantitation of Ag content by the pXRF analyzer.

Determination of Ag Content in Well-Characterized Commercial Ag Nanoparticle Samples and Commercially Available Nanoscale Ag Dietary Supplements. The reported method was also applied for the detection and quantitation of Ag content in samples that contain nanoscale Ag, such as well-characterized commercial Ag nanoparticle aqueous solutions and single- and multiple-element liquid nanoscale Ag dietary supplements. Figures 5 and 6 show representative transmission electron micrographs for these products. Particle sizes for the dietary supplements varied from a few nanometers for some individually dispersed nanoparticles to a few hundred nanometers for large particle agglomerates/aggregates.

Commercially available Ag nanoparticle aqueous solutions of three different sizes (i.e., 10, 75, and 110 nm) were analyzed with the pXRF analyzer. The results were compared to the data acquired with a method that consisted of microwave-assisted acid digestion of the nanoparticles, followed by quantitation by ICP-MS analysis. Results from both quantitation methods as well as calculated percentage difference values are shown in Table 4. Mean %DVs for the analysis of Ag nanoparticles ranged from 1.9 to 11%.

The pXRF analyzer was also used to quantify the Ag content in 12 single- and multiple-element nanobased dietary supplements, and the results are reported in Table 5. The 12 dietary supplements were selected to determine how the pXRF analyzer would perform when supplements that contain Ag levels under the MQL (i.e., DS1−DS3), as well as intermediate (i.e., DS4−DS9) and high (i.e., DS10−DS12) levels of Ag, are tested. The pXRF analyzer was able to accurately determine the concentration of Ag in the 12 nanoscale Ag dietary supplements tested, as evidenced by %DV well under ±20% for each concentration level analyzed (from near the MDL to 500 ppm). The Ag concentration values reported by the pXRF analyzer were also consistent with the concentration reported by the manufacturer on the product label for each supplement, except for DS6. For DS6, the Ag concentration values measured by the pXRF analyzer and ICP-MS are in agreement; however, the measured values are approximately 5 times higher than the manufacturer's reported value. Such discrepancies could be indicative of a labeling mistake or problems with quality control during the manufacturing of the dietary supplement.

It is also important to note that the presence of additional ingredients in the dietary supplements (i.e., proteins, stabilizers, Au, and SiO_2) did not affect the accuracy of Ag determination by the pXRF analyzer. The method's accuracy did not appear to be affected by the potential settling of large Ag nanoparticles near the sample cup film in contact with the analyzer. Caution must be taken with the analysis of large particles for long measurement times, as potential settling of the particles over time could potentially affect the accuracy of the method.

This work was undertaken to demonstrate the feasibility of using a pXRF analyzer for the rapid screening of Ag content in

Table 4. Analysis of the Well-Characterized Commercial Ag Nanoparticle (NP) Samples (n = 7)

Ag NP diameter (nm)	pXRF reported [Ag] (ppm)	ICP-MS reported [Ag] (ppm)	mean % DV
10	91 ± 3	87.1 ± 0.3	11
75	107 ± 2	105.2 ± 0.4	1.9
110	92 ± 3	85.0 ± 0.2	8.2

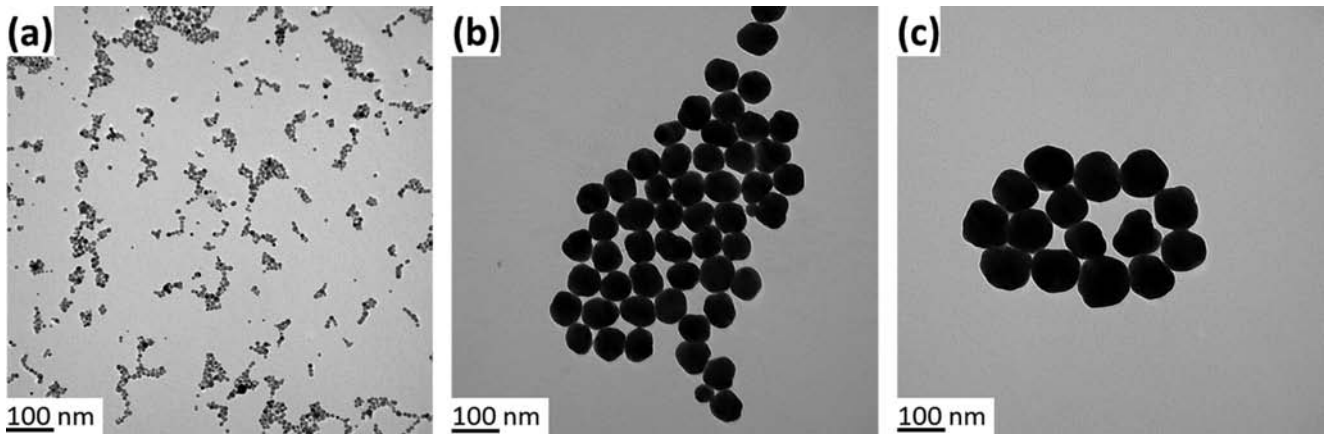

Figure 5. Representative transmission electron micrographs for well-characterized commercial Ag nanoparticles samples with nominal diameters of (a) 10 nm, (b) 75 nm, and (c) 110 nm.

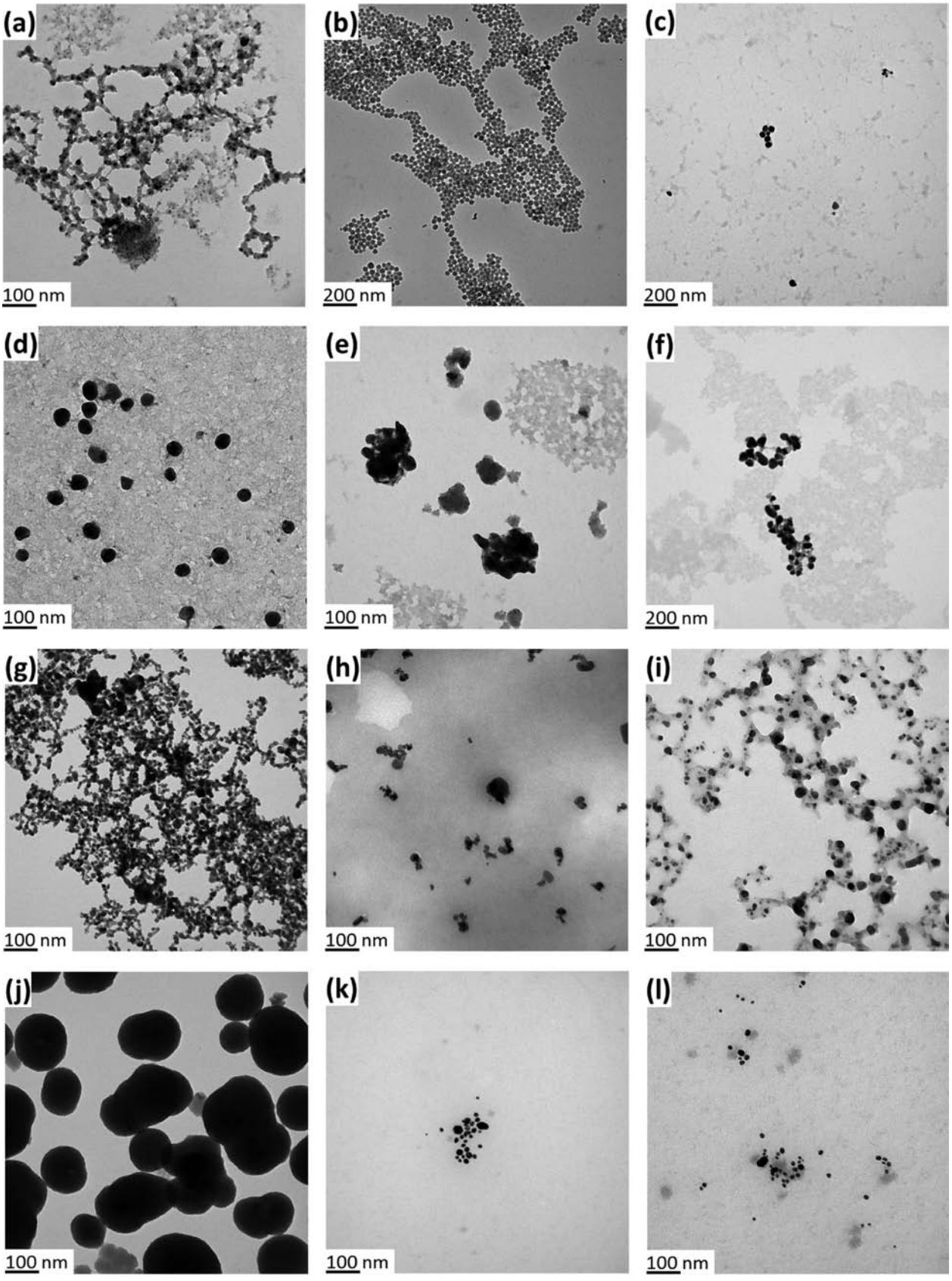

Figure 6. Representative transmission electron micrographs for commercially available nanoscale Ag dietary supplements DS1−DS12 (a−l).

FDA-regulated products that claim to contain nanoscale Ag. The results show that a pXRF analyzer is a useful tool for the rapid screening of dietary supplements that contain Ag at the ppm concentration range. The developed method allowed quick and accurate identification and quantitation of ionic Ag and Ag nanoparticles in aqueous samples, as well as in

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Table 5. Analysis of Commercially Available Nanoscale Ag Dietary Supplements (n = 7)

name	[Ag] reported on the label (ppm)	additional ingredients	pXRF reported [Ag] (ppm)	ICP-MS reported [Ag] (ppm)	mean %DV (vs ICP-MS)
DS1	3	H_2O	5 ± 1	4.76 ± 0.04	6.2
DS2	4.5	4.5 ppm Au, 500 ppm SiO_2, H_2O	7 ± 2	6.39 ± 0.03	7.6
DS3	10	H_2O	8 ± 1	7.56 ± 0.03	2.1
DS4	10	H_2O	12 ± 2	10.95 ± 0.07	13
DS5	10	H_2O	12 ± 2	11.10 ± 0.03	4.2
DS6	10	H_2O	58 ± 1	54.4 ± 0.1	5.6
DS7	20	H_2O	24 ± 1	25.20 ± 0.05	−3.8
DS8	23	H_2O	26 ± 1	23.89 ± 0.05	10
DS9	30	citric acid, H_2O	36 ± 2	34.15 ± 0.02	3.9
DS10	200	H_2O	186 ± 2	179.5 ± 0.4	3.8
DS11	250	0.1% casein, H_2O	272 ± 2	268 ± 1	1.2
DS12	500	<0.1% casein, H_2O	514 ± 3	501 ± 1	2.6

single- and multiple-element dietary supplements. The method provides a wide linear range for analysis of almost 4 orders of magnitude and MDL and MQL of 3 and 10 ppm, respectively. The MDL and MQL are suitable for detecting and quantifying Ag content in the majority of nanobased commercial dietary supplements. A random Internet-based survey of 75 commercially available liquid dietary supplements that claim to contain either nanoscale or colloidal Ag revealed that only 8% of the supplements contained Ag levels below the MQL, whereas 100% of the supplements reported Ag levels at or above the MDL. This work demonstrates that although XRF spectrometry, using portable analyzers, cannot compete with ICP-MS in terms of sub-ppm level analysis, it is very valuable for applications when speed and convenience are priorities. Its advantages in terms of ease of use, minimal sample preparation, and high-sample throughput make it an ideal technique for initial screening and field analysis of consumer products by investigators and chemists alike.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

%DV, percentage difference value; %RSD, percentage relative standard deviation; %RV, percentage recovery value; b, y-axis intercept; CCV, continuous calibration verification; CRM, certified reference material; ECV, energy calibration verification; FDA, U.S. Food and Drug Administration; IB, instrument blank; ICP-MS, inductively coupled plasma mass spectrometry; ICV, initial calibration verification; m, slope; MB, method blank; MDL, method detection limit; MQL, method quantitation limit; ppm, parts per million; PV, precision verification; pXRF, portable X-ray fluorescence; r^2, coefficient of determination; TEM, transmission electron microscopy; XRF, X-ray fluorescence

REFERENCES

(1) The Project on Emerging Nanotechnologies. http://www.nanotechproject.org/inventories/ (accessed June 1, 2012).

(2) U.S. Congress; Dietary Supplement Health and Education Act of 1994. Public Law 103-417, 108 Stat. 4325; Oct 25 , 1994.

(3) U.S. Food and Drug Administration. http://www.fda.gov/food/dietarysupplements/default.htm (accessed June 1, 2012).

(4) Musee, N.; Thwala, M.; Nota, N. The antibacterial effects of engineered nanomaterials: implications for wastewater treatment plants. *J. Environ. Monitor.* **2011**, *13*, 1164−1183.

(5) Stokinger, H. E. Silver. In *Patty's Industrial Hygiene and Toxicology*; Clayton, G. D., Clayton, E., Eds.; Wiley: New York, 1981; Vol. 2A, pp 1881−1894.

(6) Rosenman, K. D.; Moss, A.; Kon, S. Argyria: clinical implications of exposure to silver nitrate and silver oxide. *J. Occup. Med.* **1979**, *21*, 430−435.

(7) U.S. Environmental Protection Agency. Drinking Water Criteria Document for Silver (Final Draft); ECAO-CIN-026; Environmental Criteria and Assessment Office, Cincinnati, OH, 1985; PB86-118288.

(8) Hou, X. D.; He, Y. H.; Jones, B. T. Recent advances in portable X-ray fluorescence spectrometry. *Appl. Spectrosc. Rev.* **2004**, *39*, 1−25.

(9) Taylor, A.; Day, M. P.; Marshall, J.; Patriarca, M.; White, M. Atomic spectrometry update. Clinical and biological materials, food and beverages. *J. Anal. At. Spectrom.* **2012**, *27*, 537−576.

(10) Palmer, P. T.; Jacobs, R.; Baker, P. E.; Ferguson, K.; Webber, S. Use of field-portable XRF analyzers for rapid screening of toxic elements in FDA-regulated products. *J. Agric. Food Chem.* **2009**, *57*, 2605−2613.

(11) Anderson, D. L. Analysis of beverages for Hg, As, Pb, and Cd with a field portable X-ray fluorescence analyzer. *J. AOAC Int.* **2010**, *93*, 683−693.

(12) Anderson, D. L. Screening of foods and related products for toxic elements with a portable X-ray tube analyzer. *J. Radioanal. Nucl. Chem.* **2009**, *282*, 415−418.

(13) Palmer, P.; Webber, S.; Ferguson, K.; Jacobs, R. On the suitability of portable X-ray fluorescence analyzers for rapid screening of toxic elements. *FDA/ORA/DFS Lab. Inf. Bull.* **2006**, *22* (4376), 1−15.
(14) Palmer, P.; Jacobs, R. Rapid determination of chromium in stainless steel via X-ray fluorescence spectrometry. *FDA/ORA/DFS Lab. Inf. Bul.* **2007**, *23* (4398), 1−11.
(15) López-Carballo, G.; Higueras, L.; Gavara, R.; Hernández-Muñoz, P. Silver ions release from antibacterial chitosan films containing in situ generated silver nanoparticles. *J. Agric. Food Chem.* **2013**, *61*, 260−267.
(16) Zhang, Z.; Kong, F.; Vardhanabhuti, B.; Mustapha, A.; Lin, M. Detection of engineered silver nanoparticle contamination in pears. *J. Agric. Food Chem.* **2012**, *60*, 10762−10767.
(17) Martínez-Abad, A.; Lagaron, J. M.; Ocio, M. J. Development and characterization of silver-based antimicrobial ethylene−vinyl alcohol copolymer (EVOH) films for food-packaging applications. *J. Agric. Food Chem.* **2012**, *60*, 5350−5359.
(18) Mudalige, T. K.; Linder, S. W. Development of screening methodologies for the detection of nanoscale silver in FDA regulated food products using inductively coupled plasma mass spectrometry (ICP-MS). *FDA/ORA/DFS Lab. Inf. Bull.* **2012**, *28* (4514), 1−14.
(19) U.S. Environmental Protection Agency. *Field Portable X-ray Fluorescence for the Determination of Elemental Concentrations in Soil and Sediment*, 1998; Method 6200.
(20) U.S. Food and Drug Administration, Office of Regulatory Affairs. Laboratory Manual, Volume II-Methods, Method Verification and Validation ORA-LAB.5.4.5, http://www.fda.gov/ScienceResearch/FieldScience/LaboratoryManual/ucm171877.htm (accessed March 1, 2012).
(21) Bearden, J. A. X-ray wavelengths. *Rev. Mod. Phys.* **1967**, *39*, 78−124.